EVALUATION CHANGES OF LUNGS IN ASTHMATIC RATS TREATED WITH AQUEOUS AND NON AQUEOUS EXTRACT OF *CLERODENDRUM SERRATUM* AND THEOPHYLLINE.

Dissertation submitted to

THE TAMILNADU Dr.M.G.R.MEDICAL UNIVERSITY, CHENNAI-32.

In partial fulfilment of requirement for the award of the degree of

MASTER OF PHARMACY

In

PHARMACOLOGY

Submitted by

Reg.No.26119238

Under the guidance of

Dr.SORABH KUMAR AGRAWAL., M.Pharm., Ph.D.,

Associate Professor.

APRIL - 2014

DEPARTMENT OF PHARMACOLOGY,

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TAMILNADU, INDIA.

CERTIFICATE

This is to certify that the Dissertation work entitled **"EVALUATION CHANGES OF LUNGS IN ASTHMATIC RATS TREATED WITH AQUEOUS AND NON AQUEOUS EXTRACT OF *CLERODENDRUM SERRATUM* AND THEOPHYLLINE"** submitted by University Reg **No: 26119238** is a bonafide work carried out by the candidate under my guidance and submitted to the Tamil Nadu Dr. M.G.R. Medical University, Chennai, in partial fulfilment for the degree of **Master of Pharmacy** in **Pharmacology** at the Department of Pharmacology, Cherraan's college of Pharmacy, Coimbatore, during the academic year 2013-2014.

Dr.Sorabh Kumar Agrawal, M.Pharm., Ph.D.,

Associate Professor,

Department of Pharmacology,

Cherraan's college of Pharmacy.

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Dr. N. Thirumoorthy,
M.Pharm, Ph.D.,

Principal,

Cherraan's college of Pharmacy.

DECLARATION

The research work embodied in this work "**EVALUATION CHANGES OF LUNGS IN ASTHMATIC RATS TREATED WITH AQUEOUS AND NON AQUEOUS EXTRACT OF *CLERODENDRUM SERRATUM* AND THEOPHYLLINE**" was carried out by me in the department of Pharmacology, Cherraan's college of Pharmacy, Coimbatore under the direct supervision of **Dr.Sorabh kumar Agrawal M.Pharm, Ph.D.,** Associate Professor, Department of Pharmacology, Cherraan's college of Pharmacy,Coimbatore-39.

This dissertation submitted to the Tamilnadu Dr.M.G.R.Medical University, Chennai, for the award of degree of Master of Pharmacy in Pharmacology during the academic year of 2013-2014.

Place: Coimbatore.

Date: University

Reg. No **26119238**

ACKNOWLEDGEMENT

I submit my sincere thanks to our Chairman **Mr.K .C. PALANISAMY** chairman Cherraan's foundation trust, for providing all the facilities to carry out this thesis work.

With the immense pleasure and pride, we would to take opportunity in expressing our deep sense of gratitude to our beloved

guide, **Dr.Sorabh Kumar Agrawal M.Pharm., Ph.D.,**. Associate Professor, Department of Pharmacology Cherraan's college of Pharmacy under whose active guidance, innovate ideas, constant inspiration and encouragement of the work entitle "**EVALUATION CHANGES OF LUNGS IN ASTHMATIC RATS TREATED WITH AQUEOUS AND NON AQUEOUS EXTRACT OF *CLERODENDRUM SERRATUM* AND THEOPHYLLINE"** has been carried out.

My sincere gratitude to our beloved Principal **Dr. N. Thirumoorthy, M.Pharm, Ph.D.,**Cherraan's College of Pharmacy for his encouragement and also providing all facilities in this institute to the fullest possible extent enabling us to complete this work.

I convey our gratitude to **Mr.M.Gurumani, M.Pharm, (Ph.D),** Asst.Professor, Department of Pharmacology, Cherraan's College of Pharmacy, for his support for my project work.

My heartful thanks to **Mrs.Deborah Evangeline, Mr.R.Bhavani Shankar**, **Mr.Nagaraja Perumal, Dr.K.K.Senthil Kumar, Dr.P.Parthipan, Mrs.Rubina Richel, and other Teaching** and **Non-Teaching Staffs** of Cherraan's college of pharmacy for their valuable advice and co-operation.

I am giving greatful thanks to all my **Friends**, **Juniors, Seniors** & **Classmates** for their help and sharing during my project.

I express my heartfull gratitude to the **Almighty**, for giving me the right way to achieve the good for my project.

Lastly, a great thanks from my heart to my beloved **Father and Mother** .They are **my living God**, as who guided me in the rightful way to achieve all my activities. They gave me the incredible effort to become a successful person for bright future in this world. Thanks a lot to my **Parents**.

EVALUATION CERTIFICATE

This is certify that the dissertation work entitled "**EVALUATION CHANGES OF LUNGS IN ASTHMATIC RATS TREATED WITH AQUEOUS AND NON AQUEOUS EXTRACT OF *CLERODENDRUM SERRATUM* AND THEOPHYLLINE**" submitted by **Reg.No: 26119238** to The Tamilnadu Dr. M.G.R Medical University, Chennai, in the partial fulfilment for the degree of **Master of Pharmacy** in **Pharmacology** is a record of bonafide work carried out by the candidate at the department of Pharmacology, Cherraan's College of Pharmacy, Coimbatore and was evaluated by us during the academic year 2013-2014.

Internal **Examiner**

External Examiner

DEDICATED

TO

MY

BELOVED

PARENTS

Abbreviations

Abbreviations

IgE	-	Immunoglobulin E antibody
ILs	-	Interlukin's
PAF	-	Platelet activating factor
PDGF		Platelet derived growth factor
bFGF	-	Fibroblast growth factor
OECD	-	Organisation for Economic Co-Operation and Economic Development
TLC	-	Thin Layer Chromatography

Gm	-	Gram
Mg	-	Miligram
Rf	-	Resolution factor
Wt	-	Weight
Ach	-	Acetyl choline
SEM	-	Standard error of the mean
AECS	-	Aqueous extract of *clerodendrum serratum*
NAECS	-	Non aqueous extract of *clerodendrum serratum*
WHO	-	World Health Organisation
ANOVA	-	Analysis of Variance
(A)	-	Asthma group received a normal diet
(T)	-	Asthma group treated with Theophylline
(P1)	-	Asthma group which received AECS
(P2)	-	Asthma group which received AECS
(E1)	-	Asthma group which received NAECS
(E2)	-	Asthma group which received NAECS

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Former Siddha Consultant (AYUSH) Ministry of Health & Family Welfare, New Delhi.

AUT/CCP/058 13-02-2014

AUTHENTICATION

This is to authenticate that the plant ***Clerodendrum serratum*** Spr. of family **VERBENACEAE** have been collected from Nilgiri Hills Tamil Nadu, India, and handed over to Mr. G.M. Sivakumar for his project work.

ABS BOTANICAL GARDENS ★ KAARIPATTI-636 106. Salem (Dt). ★ 13/2/14

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CHAPTER-1

1-INTRODUCTION

1.1 INDIAN SYSTEM OF MEDICINE [1, 2]

The WHO estimates about 80 percentage of population living in the developing countries rely exclusively on traditional medicine for their primary health care needs. India has an ancient heritage of traditional medicine. The Indian traditional medicine is based on the different system including Ayurveda, Homeopathy, Siddha, Unani. With emerging interest in the world to adopt and study the traditional system and exploit their potentials based on the different health care system, the evaluation of the rich heritage of the traditional medicine is essential.

Almost in all traditional medicine, the medicinal plant plays a crucial role in the traditional medicine. India has the rich heritage of traditional medicine and the traditional health care system have been flourishing for many centuries. In India, the Ayurvedic system of medicine developed an extensive use of medicine from plants dating from at least 1000 B.C. Western medicine continuous to show the influence of ancient practices. For ex, cardiac glycoside from Digitalis pupurea, Morphine from Papavera samnifera, Reserpine from Rauwolfia species, Quinine from cinchona species and Arteminsin, as active anti malarial compound from Artemisia annua, etc. Show the influence of traditional medicine in the traditional medicine.

Medicinal plants and its herbal formulation are the common elements in Ayurveda, Homeopathy, Siddha and Unani system of medicines in India and China. The use of modern isolation techniques and pharmacological testing procedure means that new plant drugs usually find their way into medicine as purified substances rather than galenical preparations. The plant kingdom still holds many species of plants containing substances of medicinal value which yet to be discovered; large number of plants are constantly being screened for their possible

pharmacological value (particularly for their anti inflammatory, hypotensive, hypoglycaemic, amoebicidal, anti fertility, cytotoxicity, antibiotics, and anti parkinsonism properties).

Pharmacognosy has been basically evolved as an applied science pertaining to the study of all types of the drugs of natural origin. However, its subject matter is directed towards the modern allopathic medicine. During the course of development, many civilizations has raised and perished but the systems of medicines developed by them in various parts of the world are still practised, and are also popular as the alternative system of medicines. These are alternative systems in the sense that modern allopathic system has been globally acclaimed as the principal system of the medicine, and so all the other systems prevent and practised in various parts of the world are supposed to be alternative systems. The philosophy and the basic principles of these so called alternative systems might differ significantly from each other, but the fact cannot be denied that these systems have served the humanity for the treatment and management of disease and also for maintenance of good health. About 80 % of the world population still rely and use the medicines of traditional systems.

Traditional Chinese medicine in China, Unani system in Greece, Ayurvedic system in India, Amachi in Tibet or more recently Homeopathy in Germany are these systems of medicine which were once practised only in the respective areas or subcontinents of the world, are now popularly practised all over the world. The World Health Organization (WHO) is already taking much interest in indigenous systems of medicine and coming forward to exploit the scientific validity of the medicines used since traditions.

1.2 AYURVEDIC MEDICINES[1, 2]

Ayurvedic medicine (also called Ayurveda) is one of the world's oldest medical systems. It originated in India and has evolved there over thousands of years. In the United States, Ayurvedic medicine is considered complementary and alternative medicine. A group of diverse medical and health care systems, practices, and products that are not presently considered to be part of conventional medicine. Complementary medicine is used together with conventional medicine, and alternative medicine is used in place of conventional medicine. Complementary medicine more specifically, a CAM whole medical system. A complete system of theory and practice that has evolved over time in different cultures and apart from conventional medicine. Examples of whole medical systems include traditional Chinese medicine, Ayurvedic medicine, Homeopathy and Naturopathy. Many therapies used in Ayurvedic medicine are also used on their own as CAM for example, herbal massage, pressing, rubbing, and moving muscles and other soft tissues of the body, primarily by using the hands and fingers. The therapy increased the flow of blood and oxygen to the massaged area. This fact sheet provides a general overview of Ayurvedic medicine and suggests sources for additional information.

1.2.1 Ayurvedic Medicine in India

Ayurvedic medicine, as practiced in India, is one of the oldest systems of medicine in the world. Many Ayurvedic practices earlier written records and were handed down by word of mouth. Two ancient books, written in Sanskrit more than 2,000 years ago, are considered the main texts on Ayurvedic medicine—*Caraka Samhita* and *Sushruta Samhita*. The texts describe eight branches of Ayurvedic medicine:

- Internal medicine

- Surgery
- Treatment of head and neck disease
- Gynaecology, obstetrics, and paediatrics
- Toxicology
- Psychiatry
- Care of the elderly and rejuvenation
- Sexual vitality.

Ayurvedic medicine continues to be practiced in India, where nearly 80 percent of the population uses it exclusively or combined with conventional (Western) medicine. It is also practiced in Bangladesh, Sri Lanka, Nepal, and Pakistan.

Most major cities in India have an Ayurvedic college and hospital. The Indian government began systematic research on Ayurvedic practices in 1969, and that work continues.

Underlying Concepts

Ayurvedic medicine has several key foundations that pertain to health and disease. These concepts have to do with universal interconnectedness, the body's constitution (*prakriti*), and life forces (*doshas*).

Interconnectedness Ideas about the relationships among people, their health, and the universe form the basis for how Ayurvedic practitioners think about problems that affect health. Ayurvedic medicine holds that:

- All things in the universe (both living and nonliving) are joined together.

- Every human being contains elements that can be found in the universe.

- Health will be good if one's mind and body are in harmony, and one's interaction with the universe is natural and wholesome.

- Disease arises when a person is out of harmony with the universe. Disruptions can be physical, emotional, spiritual, or a combination of these.

Constitution (*prakriti*). Ayurvedic medicine also has specific beliefs about the body's constitution. Constitution refers to a person's general health, the likelihood of becoming out of balance, and the ability to resist and recover from disease or other health problems.

The constitution is called the *prakriti*. The *prakriti* is a person's unique combination of physical and psychological characteristics and the way the body functions to maintain health. It is influenced by such factors as digestion and how the body deals with waste products. The *prakriti* is believed to be unchanged over a person's lifetime.

Life forces (*doshas*). Important characteristics of the *prakriti* are the three life forces or energies called *doshas*, which control the activities of the body. A person's chances of developing certain types of diseases are thought to be related to the way *doshas* are balanced, the state of the physical body, and mental or lifestyle factors.

Ayurvedic medicine holds the following beliefs about the three *doshas*:

- Each *dosha* is made up of two of five basic elements: ether (the upper regions of space), air, fire, water, and earth.

- Each *dosha* has a particular relationship to bodily functions and can be upset for different reasons.

- Each person has a unique combination of the three *doshas*, although one *dosha* is usually prominent. *Doshas* are constantly being formed and reformed by food, activity, and bodily processes.

- Each *dosha* has its own physical and psychological characteristics.

- An imbalance of a *dosha* will produce symptoms that are unique to that *dosha*. Imbalances may be caused by a person's age, unhealthy lifestyle, or diet; too much or too little mental and physical exertion; the seasons; or inadequate protection from the weather, chemicals, or germs.

  The *doshas* are known by their original Sanskrit names: *vata, pitta,* and *kapha*.

  The ***vata dosha*** combines the elements Ether and Air. It is considered the most powerful *dosha* because it controls very basic body processes such as cell division, the heart, breathing, discharge of waste, and the mind. Vata can be aggravated by for example fear, grief, staying up late at night, eating dry fruit, or eating before the previous meal is digested. People with *vata* as their main *dosha* are thought to be especially susceptible to skin and neurological conditions, rheumatoid arthritis, heart disease, anxiety and insomnia.

  The ***pitta dosha*** represents the elements fire and water. Pitta controls hormones and the digestive system. A person with a *pitta* imbalance may experience negative emotions such as anger and may have physical symptoms such as heartburn within 2 or 3 hours of eating. *Pitta* is upset by for example eating spicy or sour food, fatigue, or spending too much time in the sun. People with a predominantly *pitta* constitution

are thought to be susceptible to hypertension, heart disease, infectious diseases, and digestive conditions such as Crohn's disease.

The ***kapha dosha*** combines the elements water and earth. *Kapha* helps to maintain strength and immunity and to control growth. An imbalance of the *kapha dosha* may cause nausea immediately after eating. *Kapha* is aggravated by for example, greed, sleeping during the daytime, eating too many sweet foods, eating after one is full, and eating and drinking foods and beverages with too much salt and water (especially in the springtime). Those with a predominant *kapha dosha* are thought to be vulnerable to diabetes, cancer, obesity, and respiratory illnesses such as asthma.

1.3 UNANI SYSTEM OF MEDICINE[1, 2]

Unani system of medicine is originated in Greece by the Greek philosopher, physician Hippocrates (460-377 B.C), who freed medicine from the realm of superstition and magic, and gave it the status of science. The theoretical frame work of Unani medicine is based on the teachings of Hippocrates. After him, a number of other Greek scholars followed the system consider. Among them Galen (131-212 A.D) was one to stabilize its foundation, on which Arab physicians like Razzes (850-925 A.D) and Avicenna (980-1037 A.D) constructed as imposing edifice. Unani medicine got its importance among the other systems of medicine in Egypt, Syria, Iraq, Persia, India, China and other Middle East and Far East countries. In India, Arabs introduced Unani system of medicine, and soon it enriched in India.

Unani medicine is based on the Greek philosophy. According to basic principles of Unani, the body is made up of four basic elements, i.e. Earth, Air, Water and Fire, which have different Temperaments, i.e. Cold, Hot, Wet and Dry. After mixing and interaction of four elements, a new compound having new temperament comes into existence, i.e. Hot Wet, Hot Dry, Cold Wet and Cold Dry. The body has the simple and compound organs, which got their nourishment through

four humours, i.e. blood, phlegm, yellow bile and black bile. The humour also assigned temperament as blood i.e. hot and wet; Phlegm is cold and dry. Health is a state of body in which there is equilibrium in the humours and functions of the body are normal in accordance to its own temperament and the environment.

When the equilibrium of the humours is disturbed and functions of the body are abnormal, in accordance to its own temperament and environment, that state is called disease. Unani medicine believes in promotion of health, prevention of disease and cure. Health of human is based on the six essentials (Asbabe sitta Zaroorya), if these are followed health is maintained; otherwise, there will be disease. Six essentials are atmospheric air, drinks and food, sleep and wakefulness, excretion and retention, physical activity and rest and mental activity and rest.

1.4 HOMEOPATHIC SYSTEM OF MEDICINE [2]

Homeopathy is a specialised system of therapeutics, developed by Dr Samuel Christian Friedrich Hahnemann (1755- 1843), a general physician, chemist and a pharmacist, based on natural law of healing: similia similibus curantur, which means 'Likes are cured by likes'. Homois means like (similar) and pathos means treatment. Thus, Homeopathy is a system of treating diseases or suffering by the administration of the drugs that possess power of producing similar suffering (disease) in healthy human beings. Dr Hahnemann believed that symptoms are no more than an outward reflection of the body's inner fight to overcome illness: it is not a manifestation of the illness itself. This law of similar for curing diseases has being in use since the time of Hippocrates, father of medicine. Dr Hahnemann who developed it in to a complete system of therapeutics enunciating the law and its application in 1810.

1.4.1 FUNDAMENTAL PRINCIPLES OF HOMEOPATHY [1, 2]

Homeopathy as a science of medical treatment has a philosophy of its own, and its therapeutics is based on certain fundamental principles that are quite distinct and different between from those of other school of medical science. These fundamental principles were discussed by Hahnemann in different sections of his medicine and philosophy.

They are as follows:

1. Law of similia.
2. Law of simplex.
3. Law of minimum.
4. Drug proving.
5. Drug dynamization or potentiation.
6. Vital force.
7. Acute and chronic disease.
8. Individualization.
9. Direction of cure.

1.5 AROMATHERAPHY [1, 2]

The word Aromatheraphy means treatment using scents. It refers to the use of essential oils in Holistic healing it improve health and emotional well being, and in restoring balance to the body. Essential oils are aromatic essences extracted from plants, flowers, trees, fruit, bark, grasses and seeds. There are more than 150 types of oils that can be extracted. These oils have distinctive therapeutic, psychological and physiological properties that improve health and prevent illness. All essential oils have unique healing a valuable anti septic properties. Some oils are

anti viral, anti inflammatory, anti pain relieving, antidepressant, stimulating, and relaxing, expectorating, support digestion and have diuretic properties too.

Essential oils get absorbed into our body and exert an influence on it. The residue gets dispersed from the body naturally. They can also affect our mind and emotions. They enter the body in three ways: by inhalation, absorption and consumption. Chemically, essential oils are a mixture of organic compounds like ketones, terpenes, esters, alcohol, aldehyde and 100 of other organic molecules which are extremely difficult to classify, as they are small and complex. The essential oils molecules are small they penetrate human skin easily and enter the bloodstream directly and finally get flushed out through our elementary system.

Some of the common essential oil used in Aromatherapy

1. Clary sage (saliva scared)
2. Eucalyptus (eucalyptus globules)
3. Geranium (pelargonium graveolens)
4. Lavender (citrus lemon)
5. Peppermint (mentha pipertia)

1.6 SIDDHA SYSTEM OF MEDICINE [2]

Siddha medicine is practised in southern India. The origin of the Tamil language is attributed to the sage Agasthya, and the origin of Siddha medicine is also attributed to him. Before the Aryan occupation of Sind region and the Gangetic plain, there existed in the southern Indian, on the banks of the river Cauvery and Tamiraparani, a civilization which was highly organized.

1. This civilization has the system of medicine to deal with problem of sanitation and treatment of disease. This is the Siddha system of medicine. The therapeutics of Siddha medicine consists mainly of the use of metals and minerals where as in the earlier Ayurveda.

2. There is mention of mercury, sulphur, copper, arsenic and gold used a therapeutic agents.

1.6.1 PRINCIPLE OF SIDDHA SYSTEM OF MEDICINE [2]

The universe consists of to essential entities: matter and energy the Siddha called them Siva (male) and Sakthi (female, creation). Matter cannot exist without energy inherent in it and vice versa. The two co exist and are inseparable. They are the primordial elements (Buddha's) and are not be confused with modern chemistry. Their names are Mann (solid), Neer (fluid), Thee (radions), Vaayu (gas) and Agasum (ether). These five elements (Buddha's) are present in every substance, but in different proportions. Earth, water, fire, air and ether are manifestation of five elements.

The human beings made up these five elements indifferent combination, the physiological function in the body is mediated by three substances (dravyas), which are made up of the five elements. They are vatham, pitham and karpam. In each and every cell of the body these three doshas coexist and function harmoniously.

The seven dhatus are as follows:

1. Rasa (lymph).
2. Kurudhi (blood).
3. Tasai (muscle).
4. Kozhupu (adipose tissue).
5. Ezhumpu (bone).
6. Majjai (marrow).
7. Sukkilam and Artavam (male and female hormones)

CHAPTER 2

2. ASTHMA REVIEW[3, 4, 5]

Asthma is a hyper reactive airway disease running a chronic course; it has worldwide prevalence and is a common cause hospitalization in children. It is estimated that currently 300 million peoples suffer from asthma with a possibility of an additional 100 million likely to suffer from the disease over the next 15-20 years.

Asthma is a chronic inflammatory disorder of the airway in which many cells and cellular elements play a role, in particular, mast cells, eosinophils, T-lymphocytes, macrophages, neutrophils and epithelial cells. In susceptible individuals, this inflammation causes recurrent episodes of wheezing, breathlessness, chest tightness and coughing, particularly at night or in the morning. These episodes are usually associated with widespread but variable airflow obstruction that is reversible either spontaneously or with treatment. The inflammation also causes an associated increase in the existing bronchial responsiveness to a variety of stimuli.

2.1 ETIOLOGY

There are two types of asthma; 1. Allergic or extrinsic 2. Idiosyncratic or intrinsic. Allergic asthma is a result of an antigen/antibody reaction on mast cells in the respiratory tract. This type is often associated with a family history of atopy (allergic diseases) such as eczema. Idiosyncratic asthma or intrinsic asthma is a result of neurological imbalances in the autonomic system in which the system in which the sympathetic and parasympathetic systems are not properly coordinated.

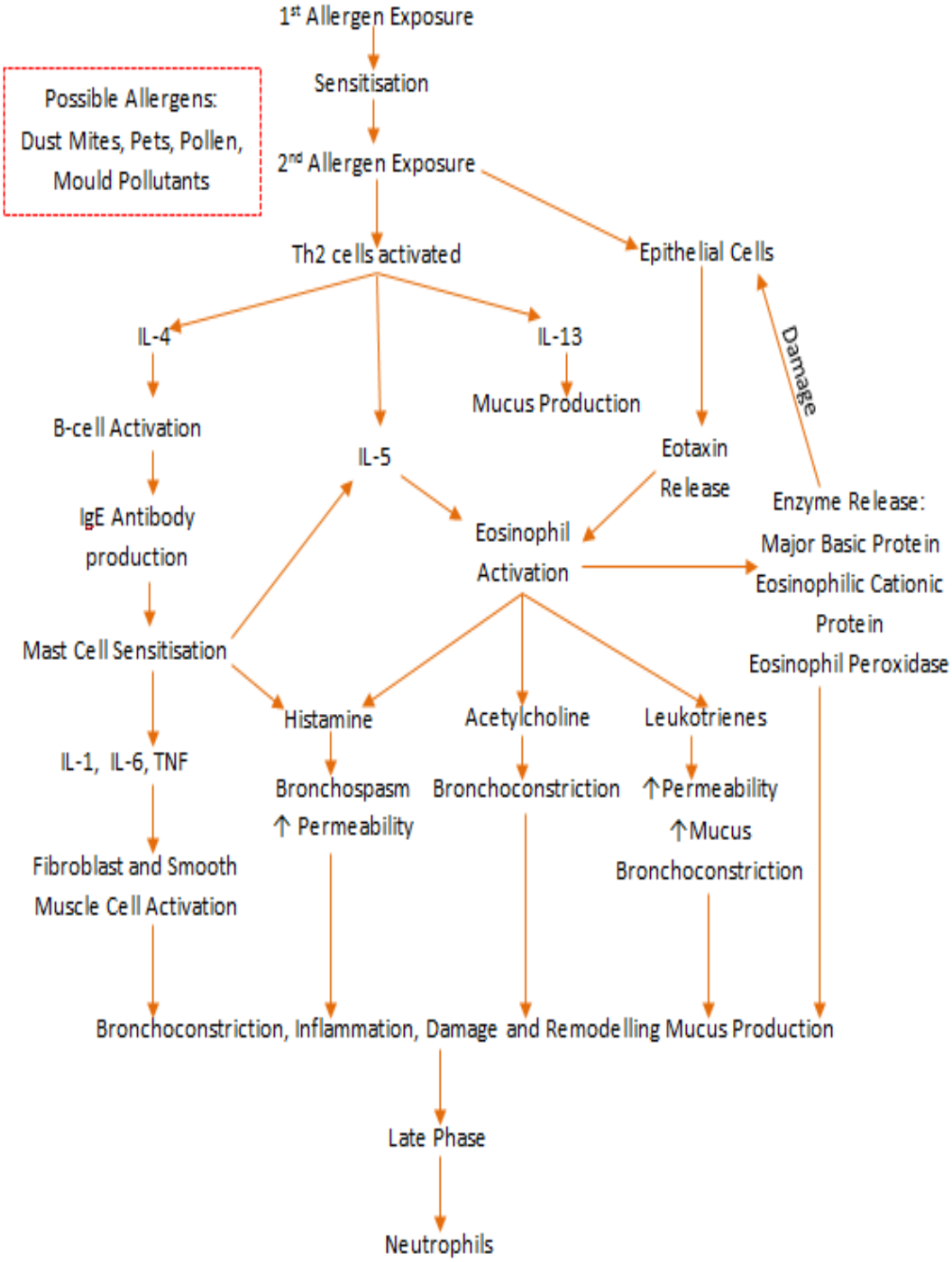
The causes for asthma are many including environmental and genetic factors. Atopy is the strongest genetic predisposition for the development of an IgE response to common aeroallergens. Most cases of childhood asthma (90%)

are allergic in nature; it is referred to as extrinsic asthma. In this form of the disease, the inflammatory reaction is a result of abnormal response to common aeroallergens and environmental allergens. The late includes dust mites, animal, pollen, moulds, fungi, cockroaches, fossil fuels, strong fumes, inhalation of irritants (sprays and paints), cigarette smoke and exposure to cold and humid weather. The environmental factors act as triggers in those persons who have inflammatory changes in their respiratory passages and are highly susceptible to acute attacks of asthma. The other important causes of asthma includes viral respiratory infections, exercise (hyperventilation), chronic sinusitis or rhinitis, gastroesophageal reflux disease (GERD) and drug-induced asthma (aspirin,NSAIDs,β blockers, cholinergic drugs). In exercise induced asthma, the contributing factors include exposure to cold or dry weather and environmental pollutants (sulphur, ozone).

2.2 PATHOPHYSIOLOGY

Chronic inflammatory reaction is the characteristic features in the pathogenesis of bronchial asthma which results in intermittent airflow obstruction and bronchial hyper responsiveness. The airway obstruction in asthma is due to factors that includes bronchospasm oedema of the airway, increased mucus secretion, cellular infiltration of the airway walls and injury to the airway epithelium. Repetition of the inflammatory events in asthma can cause irreversible functional changes in the airway passages, a process called remodelling. The remodelled airway passages are persistently narrow as the disease progresses and become less and less responsive to drug treatment. The mechanism of inflammation in asthma may be acute, sub acute or chronic.

Pathogenesis of Asthma



2.3 INFLAMMATORY REACTION

An attack of asthma begins when the allergens is inhaled. The allergen binds to IgE antibodies that have binding side for the allergens mast cells in the lungs. Binding of allergen to IgE triggers exocytosis of the mast cells with the release of histamine leukotrienes. Leukotrienes cause the smooth muscle cells of the bronchi to contract, narrowing the lumen of the bronchi. This is the early phase. Next, they attract on accumulation of the inflammatory cells, especially eosinophils-leading to production of mucus. This is the late phase which occurs 4-6hrs after the early phase.

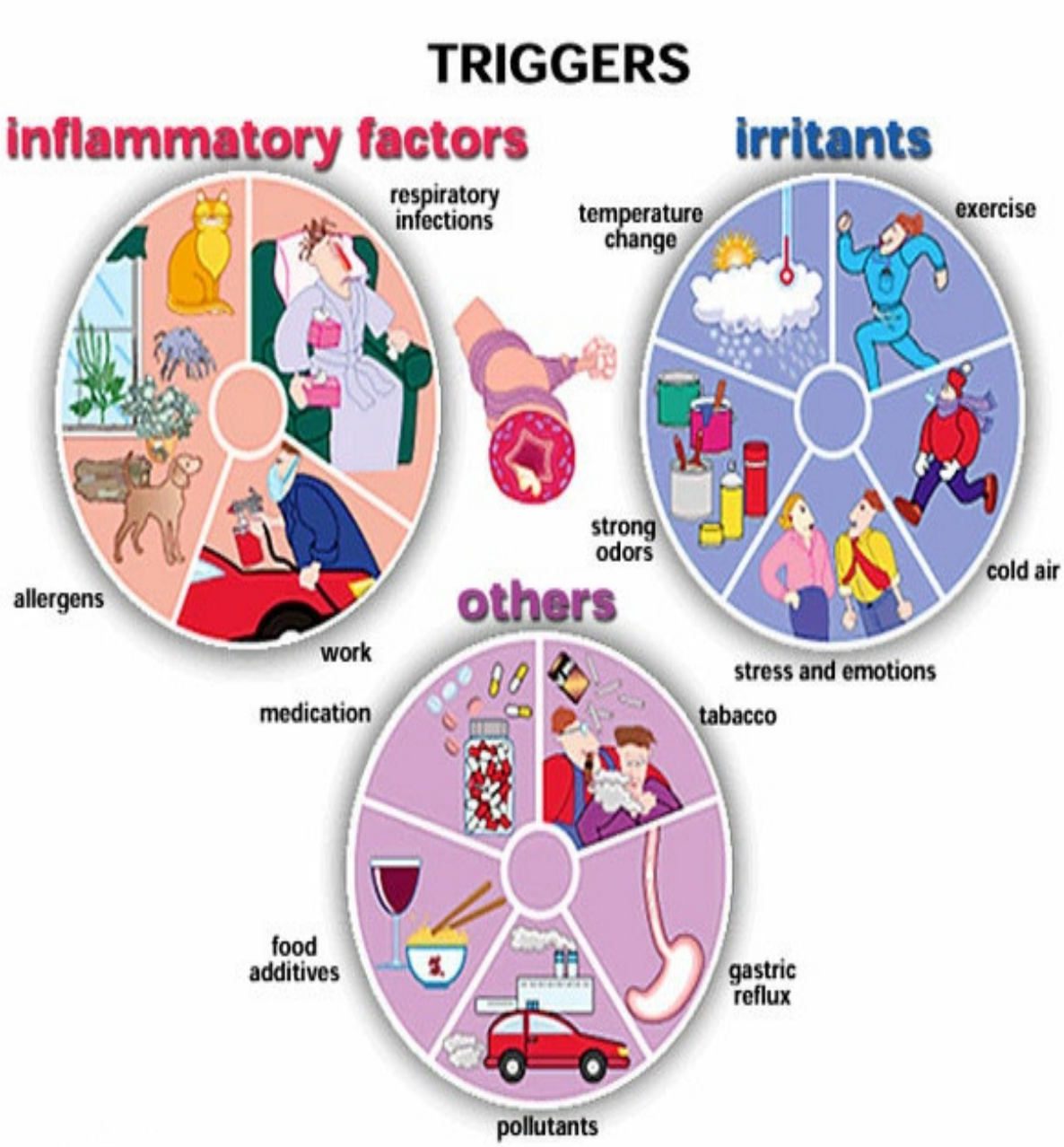
- Activation of helper T cells, in particular, a sub group called Th_2 cells (CD_4+T cells producing Th_2)
- Th_2 cells over produce interleukins (ILs)
- Interleukins 4, 5, 9 and 13may be responsible for a first phase asthma attack. These interleukins stimulate the release IgE. People with both asthma and allergies appear to be predisposed to over production of IgE.
- During an allergic attack, these IgE antibodies can bind to mast cells present in the lungs, and also in this skin and mucus membranes. This binding triggers the release of histamine and leukotrienes which are responsible for the airway spasms.
- Another cytokine, IL5, appears to contribute to late phase inflammatory reaction. This ILs releases Eosinophils. These cells accumulate and remind in the airways after the first attack. Activation of Eosinophils initiates contraction of bronchial smooth muscles, increased micro vascular permeability, induction of airways hyper responsiveness and mucus production. The pathological changes in asthma and the mediators responsible are summarised.

TABLE:1

Pathological changes	Mediator responsible
Contraction of bronchial smooth muscle causing bronchospasm	Histamine(H_1 receptor – mediated action), leukotrienes C_4,D_4,E_4, prostaglandins & TXA_2, bradykinin, platelet activating factor (PAF)
Mucosal oedema	H_1 receptor mediated action, leukotrienes C_4,D_4,E_4, prostaglandins & bradykinin, platelet activating factor (PAF)
Airway hyper reactivity	Anaphylaxis, Eosinophil chemotactic factors, neutrophil chemotactic factors, LTB_4, PAF.
Mucus secretion	Histamine (H_1 receptor – mediated action), leukotrienes C_4, D4, E4, prostaglandins & TXA_2, bradykinin, platelet activating factor (PAF), Eosinophils.

Inflammatory cells released in asthma include mast cells, degranulation of which causes release of histamine; eosinophils which initiate contraction of bronchial smooth muscles, increase microvascular permeability and induce airway responsiveness, neutrophils having a passive effector role in inflammation via phagocytosis and release of performed enzymes and cyto toxic compounds; and macrophages which play a major role in the pathogegesis of injury and tissue repair and are involved in airway remodelling through secretion of growth factors such as platelet derived growth factor (PDGF), and basic fibroblast growth factor (bFGF) of TGF – B.

2.4 INFLAMMATORY MEDIATORS:


TRIGGERS
inflammatory factors
irritants
respiratory infections
temperature change
exercise
allergens
strong odors
cold air
work
stress and emotions
others
medication
tabacco
food additives
gastric reflux
pollutants

2.5 SYMPTOMS:

- ✓ Chest pain,
- ✓ Wheezing,
- ✓ Cough,
- ✓ Difficult to breath, etc.,

2.6 INFLAMMATORY REACTION IN BRONCHIAL ASTHMA

Early phase → activation of Th_2 cells → interleukins 4,9,13 production ↑

↓

Levels of Ig E ↑

↓

Late phase — Allergen →binding of IgE to the mast cells

↓ — ↓

Release of IL_5 — Release of histamine and leukotrienes

↓ — ↓

Eosinophils ↑ — Bronchoconstriction, bronchospam

↓ — ↓

Activation of Eosinophils — hyper reactivity ↑

Activation of neutrophils — ↓

Activation of macrophages — Attack bronchial asthma

2.7 TREATMENT FOR ASTHMA:

1. SYMPATHOMIMETIC AGENTS:

- ✓ Salbutamol
- ✓ Terbutaline
- ✓ Metaproterenol
- ✓ Fenoterol
- ✓ Bitolterol
- ✓ Pirbuterol
- ✓ Salmeterol
- ✓ Formoterol

2. METHL XANTHINE DERIVATIVES:

- ✓ Theophylline
- ✓ Aminophylline
- ✓ Choline theophyllinate
- ✓ Hydroxyethyl theophylline
- ✓ Theophylline ethanolate of piperazine
- ✓ Doxophylline

3. ANTI CHOLINERGIC:

- ✓ Ipratropium bromide
- ✓ Tiotropium bromide

4. LEUKOTRIENE ANTAGONISTS:

- ✓ Montelukast
- ✓ Zafirlukast
- ✓ Ziluten

5. MAST CELL STABILIZERS:

- ✓ Sodium cromoglycate
- ✓ Ketotifen

6. CORTICOSTEROIDS:

❖ SYSTEMIC:

- ✓ Hydrocortisone
- ✓ Prednisolone
- ✓ Methyl prednisolone
- ✓ Dexamethasone

- ❖ INHALATION:
- ✓ Beclomethasone dipropionate
- ✓ Budesonide
- ✓ Fluticasone dipropionate
- ✓ Mometasone fumarate
- ✓ Cicleoside
- ✓ Triamcinolone
- ✓ Flunisolide

7. ANTI IgE ANTIBODY:

- ✓ Omalizumab

2.8 CLINICAL MANAGEMENT OF ASTHMA:

Bronchial asthma is a chronic debilitating disease associated with morbidity and mortality. Judicious management of the disease is essential depending upon the severity of the disease. Treatment of childhood asthma requires special attention as children are very vulnerable and present with varying degrees of clinical features often requiring regular visits to hospital. A general guideline stipulates that the smallest dose of the drug needed to adequately control the symptoms should be used. Patients with more frequent or severe symptoms or with impaired lung function should be treated with regular prophylactic therapy such as inhaled glucocorticoids, if necessary in the higher doses depending on the severity of the symptoms. Long acting β_2 agonists such as salmoterol and formoterol may be added to assist rapid control of symptoms. Leukotriene modifying agents such as montelukast are quiet helpful in children

for prophylactic use to prevent exacerbations of asthma likely to occur when they are exposed to known and unknown allergens including environmental allergens. One important consideration for the therapy of asthma is the possibility of the drug tolerance and drug toxicities which may limit their usefulness and require a change in the drug.

3-LITERATURE REVIEW

***Mukesh Kr. Singh et al.*[6]**

The chemical constituents such as carbohydrates, flavonoids, phenolics, steroids, and terpenes were found so while administered to the rats at 100mg/kg and 200mg/kg of Aqueous extracts of leaves of *Clerodendrum serratum* possess bronchodilator property.

***Jatin Sharma et al.*[7]**

Ayurvedic science has propagated the use of *Clerodendrum serratum* as effective treatment against asthma, bodyache, cholera, eye disorder, ulcers, snake-bite, wound, tuberculosis and epilepsy. Stimasterol, α-spinasterol, luteolin, luteolin-7-0 glucuronide, apigenin, baicalin and scutellarin 7-0 glucuronide are found in leaf based upon the above molecules steroids and flavonoids used for anti asthmatic activity.

***Neeta Shrivastava Tejas Patel et al.*[8]**

C.serratum Leaves are reported to be used as medicine for the treatment of asthma, pyreticosis, cataract, malaria, and diseases of blood, skin and lung. To prove these ethno-medical claims, some of these species are being extensively studied for their biological activities using various animal models. Along with biological studies, isolation and identification studies of chemical constituents and its correlation with the biological activities of the genus has also been studied. The major chemical components reported from the genus are phenolics, steroids, di- and triterpenes, flavonoids and volatile oils.

***Farah Farokhi et al.*[9]**

Plantago major(P. major) is one of the medicinal crops in the world which has therapeutic properties for treatment of respiratory and gastrointestinal diseases. Theophylline is commonly used for the treatment of respiratory diseases. In this study, we investigated the protective effects of hydro-alcoholic

extract of *P. major* on lung in asthmatic male rats. in asthmatic group. The mean number of mast cells was significantly increased ($p<0.05$). Thickness of alveolar epithelium and accumulation of glycoprotein in airways was increased. Moreover, in some of alveolar sac haemorrhage was observed. Administration of *p.major* extract in asthmatic rats restored these changes towards normal group.

***Ziad Shraidehet al.*[10]**

This study is an attempt to reveal the effects of narghile smoking on the cellular level, through exposing a group of experimental albino rats to the smoke of two types of narghile tobacco-derived products: flavored (moassal) and unflavored (tumbak), for three months on a daily basis, using a specially designed smoking machine. The most prominent histological changes were an abnormal proliferation in the epithelium of trachea, disruption of its cilia, and a marked hyperplasia in the connective tissue of lung alveoli.

***Surendra Adusumalli et al.*[11]**

Treatment with aqueous extract of *Pistacia Integerrima* galls showed a dose dependent effect on disruption rate of actively sensitized mesenteric mast cells of albino rats when challenged with antigen (horse serum along with triple antigen vaccine).Aqueous extract of galls treatment for ten days resulted in significant protection against histamine aerosol-induced bronchospasm in guinea pigs and showed the spasmolytic activity against histamine induced contractions in isolated guinea pig tracheal chain preparation.

***Bhoomika R. Goyal et al.*[12]**

We have studied the bronchoprotective effect of ethanolic extract of *Achyranthes aspera* Linn. in toluene disocyanate (TDI) induced occupational asthma in wistar rats. TDI sensitized rats exhibited asthmatic symptoms while A. As per and dexamethasone treated rats did not show any airway abnormality. The neutrophils and eosinophils in blood were decreased significantly; the total cells

and each different cell in particular eosinophils in BAL fluid were markedly decreased in treatment groups as compared to TDI sensitized rats. The antioxidant activity and histopathological observations also showed protective effect.

***S.M. Vidya et al.*[13]**

The root bark extracts of *Clerodendrum serratum* contains D-mannitol, stigmasterols, three triterpenoids such as oleanolic acid, queretaric acid and cerratagenic acid (Banarjee et al. 1969). The plant was investigated for its potential activity viz.,anti-inflammatory, analgesic, antipyretic activity.

***Abeer A.A. Salama et al.*[14]**

To evaluate the efficacy of fish oil (FO) alone or combined with half the dose of dexamethasone (DEX) in experimentally-induced bronchial asthma Asthma was induced in the remaining groups by ovalbumin (OVA) sensitization (1 mg/kg OVA; i.p.) for 3 consecutive days followed by 1% OVA challenge (1 day/week for 3 weeks). One group was left untreated (positive control). In the remaining groups, test agents were orally administered 1 h before each OVA challenge as follows: group 3 received dexamethasone (DEX; 1 mg/kg), groups 4-6: received FO (1, 2 and 3 g/kg) and group 7 received FO (1.5 g/kg) plus DEX (0.5mg/kg). Lung function tests were assessed 12 min after the last OVA challenge and 24 h thereafter,blood films were prepared for assessment of eosinophil count and blood samples were collected for assessment of serum total protein as well as immunoglobulin E (Ig-E) levels. Lungs were isolated for histopathological assessment and determination of tumor necrosis factor-alpha (TNF-α) content. Additionally the effects of test agents were evaluated in acetyl choline (ACh; 0.003-0.03%)-induced airway constriction. Results FO alone and combined with DEX attenuated OVA-induced changes in lung function tests, reduced OVA-induced increase in eosinophil count, serum total protein and Ig-E

Levels as well as lung TNF-α content and reduced airway remodelling. Moreover, FO and DEX inhibited ACh-induced airway constriction.

Conclusions: FO can be used alone or combined with a lower dose of DEX in treatment of bronchial asthma.

***Neelmani Chauhan et al.*[15]**

Treatment with alcoholic extract of *clitorea ternatea* (400 mg/kg, p.o) showed significant protection against histamine aerosol induced bronchospasm in Wister rats. The results of the histamine- induced bronchospasm paradigms demonstrate bronchospasmolytic activity of ethanolic extract of *clitorea ternatea* (400 mg/kg, p.o.). *Clitorea ternarea* shows 47.45 % protection against histamine induced bronchoconstriction in rats.

***DJ Taur et al.*[16]**

In present study ethanol extract of *Abrus precatorius* leaves (EAPL) at doses of 100, 125, 150mg/kg i.p were evaluated for preliminary phytochemical screening, acute toxicity studies and egg albumin induced mast cell degranulation in mice and passive cutaneous anaphylaxis in rats.the results of present investigation showed that the LD_{50} of EAPL is more than 300 mg/kg. EAPL (100-150mg/kg, i.p.) significantly protect egg albumin induced degranulation of mast cell and inhibit area of leakage of dye in passive cutaneous anaphylaxis. Phytochemical studies observed presence of saponin, alkaloids, flavonoids, and glycosides. In conclusion EAPL possesses anti asthmatic potential.

***Priyashree Sunita et al.*[17]**

The effect of Fr-Et and Fr-Me were studied on acetylcholine and histamine aerosol-induced broncospasm using guinea pigs as experimental animals. Also, the effects of these fractions were evaluated on the isolated guinea pig tracheal preparations. Besides this mast cell degranulation effect was assessed using egg albumin and compound 48/80on rat peritoneal mast cells. Significant increase in preconvulsion time was observed due to pre-treatment with the fractions when guinea pigs were exposed to histamine and acetylcholine

aerosol. Fr-Et and Fr-Me significantly increased the preconvulsion in a dose depended manner that suggestive of bronchodilating activity.

***Dnyaneshwar J Taur et al.*[18]**

Ethanol extracts of *C. grandis* fruit (ECGF) at 100,125 and 150 mg·kg, i.p., was evaluated for mast cell stabilizing, anti anaphylactic and antihistaminic activity using egg albumin induced mast cell degranulation in mice; passive cutaneous anaphylaxis in rats and clonidine induced catalepsy in mice respectively. ECGF at (100−150 mg·kg, i.p.) significantly protected egg albumin induced degranulations of mast cells and caused reduction of blue dye leakage in passive cutaneous anaphylaxis in dose dependently. The treatment ECGF also inhibited clonidine induced catalepsy in dose dependent manner. Phytochemical studies observed presence of saponin, steroids, alkaloids, flavonoids and glycosides. In conclusion ECGF possesses mast cell stabilizing; anti anaphylactic and antihistaminic potential which might be used in treatment of asthma.

***Afreen Ansari et al.*[19]**

In vivo models: Inhibition of histamine induced broncho constriction by various doses of test compound and standard is recorded. ED_{50} values for inhibition in pulmonary resistance (RL) are calculated. Furthermore, the time course of histamine antagonism can be evaluated. Compounds can be tested either after IV injection of histamine (prevention) or during intravenous infusion of histamine (intervention).

***M.S.Harisha et al.*[20]**

The ethanolic and aqueous extracts of the whole plant of *S.orobanchioides* were evaluated for antihistaminic and mast cell stabilizing activities. Both extracts inhibited histamine-induced contractions of the guinea-pig ileum at the concentration range of 2.5– 25 g/ml in a dose-related manner. At 25g/ml, both extracts inhibited the response of histamine (0.5g/ml) almost completely. The effect of these two extracts on the degranulation rate of

sensitized peritoneal cells of albino rats when challenged with antigen (horse serum) was studied. Triple vaccine was used as adjuvant. Ketotifen and prednisolone were used for comparison. The ethanolic extract at 100 and 200 mg/kg body weight was found to significantly inhibit degranulation of mast cells to an extent of 52.143 and 67.963 respectively. At the same doses, the aqueous extract showed 42.092.91 and 60.673.50% reduction in degranulation of mast cells, respectively.

P. Venkatesh et al. [21]

Estimation of histamine release is key parameter for evaluating any target for its anti-allergic potential. The stabilization potential of the alcoholic extract of COR (100–400 mg/kg) against mast cell degranulation was studied on isolated mice peritoneal mast cells. The antihistaminic activity was performed by determining the mortality rate of mice upon exposure to compound 48/80 and effecton inhibition of histamine release upon degranulation. The raised number of intact mast cells intimates that the COR stabilized the mast cell degranulation (60.96±1.96%) and percentage antihistaminic potential of the extract (63.58±1.8 inhibition at dose of 400 mg/kg) and it virtues further work towards the isolation of phytoconstituents from this plant. This finding provides evidence that COR inhibits mast cell-derived immediate-type allergic reactions and mast cell degranulation.

4-AIM OF STUDY

Asthma is one of the common disorders encountered in clinical medicine in both adults and children are asthma and it is characterized by inflammation of the airways which causes airway dysfunction.

Asthma is currently a worldwide problem with around 300 million people around the global suffering from it and world deaths of about 25000 annually. Inhaled bronchodilators and anti inflammatory drugs are available and effective and they require long term use and are associated with side effects.

This is why alternative and complementary medicine is being sort after to prevent these side effects.

Several medicinal plants have anti inflammatory effect and have proved effective in the treatment of asthma.

Clerodendrum serratum Linn (Family-Verbenaceae) is very widely distributed in tropical and subtropical regions of the world.

Citric acid, Acetylcholine, Histamine is mainly used for evaluating anti asthmatic activity of particular drug. This review contains list of medicinal plant which have been tested for anti asthmatic activity in the Citric acid, Acetylcholine induced asthmatic in Rat model

Thus, the information provided in this research will help the researchers for the development of an alternative methods rather than inhalers and oral anti asthmatic drugs for the treatment of asthma and COPD which will minimize the complication.

Many plants obtained from the natural source play a significant role in the health care system.

Literature survey on herbal drugs has shown significant anti asthmatic activity which has not shown any remarkable side effect. The pharmacological

mechanism which the phyto constituents producing the anti asthmatic activity are not clearly understood till date. The several herbal formulation have derived from the Ayurveda, traditional system of Indian medicine and its additional system of medicine, yet to be scientifically validated that they have exhibited pharmacological action against Asthmatic. Only less number of scientific data of traditional medicines is available for the treatment of Asthmatic.

5-PLANT PROFILE [22,23,24]

Synonym: *Rotheca serrata* (L.) Steane & Mabb.

5.1 CLASSIFICATION [22,23,24]

Kingdom : Plantea

Class : Magnoliopsida

Subclass : Lamiidae

Order : Lamiales

Family : Lamiaceae/ Verbenaceae

Sub-family : Ajugoideae

Genus : *Clerodendrum*

Species : *serratum*

5.2 VERNACULAR NAMES

English : Blue glory, Beetle killer

Hindi : Bharangi

Kannada : Gantubarangee

Malayalam : Cheruthekku

Sanskrit : Angaravalli, Padma, Brahmanayashtika, Barbura

Tamil : Cheruteku

Telugu: Ganttubrarangee

Urdu : Bharangi, Baharangi

5.3 DISTRIBUTION:

Clerodendrum serratum Linn native to tropical and warm temperate regions of the world, with most of the species occurring in tropical Africa and southern Asia, but some in the tropical Americas and northern Australia, and a few extending north into the temperate zone in eastern Asia.

5.4 DESCRIPTION:

Clerodendrum serratum Linn (Verbenaceae).is a slightly woody shrub with blunty stems and branches. These trees are about 2-8 ft high. It is annual or perennial, usually aromatic.

5.5 CHEMICAL CONSTITUENTS:

Leaf extracts contains

- ✓ Stigma sterol,
- ✓ A-spinasterol,
- ✓ Luteolin,
- ✓ Luteolin-7-0 glucuronide,
- ✓ Apigenin,
- ✓ Baicalin
- ✓ Suctellarin 7-0 glucuronide.

5.6 PARTS USED:

Fresh leaves, flowers and dried fruits, roots, seeds, stem, barks.

5.7 TRADITIONAL USE:

Clerodendrum serratum as effective treatment against asthma, bodyache, cholera, eye disorder, ulcers, snake-bite, wound, tuberculosis and epilepsy. It has antibacterial, antihistaminic, hepatoprotective, antipyretic, antinociceptive and anti-inflammatory.

Root is pungent, bitter, acrid, dry, heating, anti-inflammatory, digestive, carminative, depurative, expectorant, antispasmodic, stimulant, appetizer and anthelmintic.

Leaves are used in fever and hiccough. Its boiled leaves are used in cephalgia and opthalmia where as its boiled seeds in butter milk is used as aperients, in dropsy and in catarrhal affection of lungs (Shah, 2003).

*Clerodendrum serratum,*its methnolic extract exhibit significant anticancer activity as compared to aqueous extract. (Zalke et al. 2010).Antibacterial activity of *Clerodendrum serratum .L* (Vidya S.Met al. 2010).

6-PLAN OF WORK

Objectives:

The present study is to evaluate the anti asthmatic activities of Leaves in *Clerodendrum serratum* Linn.

Step: 1

Plant collection and authentication of plant species.

Step: 2

Preparation of plant extracts.

- Aqueous extract of *clerodendrum serratum.*
- Ethanolic extract of *clerodendrum serratum.*

Step: 3

- Evaluations of phytochemical constituents.
- Estimation of active constituents by TLC method.

Step: 4

Acute toxicity studies as per OECD guidelines.

Step: 5

Anti Asthmatic activity:

- ✓ Evaluation changes of lungs in asthmatic rats treated with Aqueous and non aqueous extract of *clerodendrum serratum* and theophylline.

Sacrifying animals and giving for Histopathological studies.

7-MATERIALS AND METHODS

7.1 COLLECTION OF PLANT

A novel approach to plant selection is a computerized selection method or literature information selection technique (LIST) that correlates biological activity, botanical facts and chemotoxanomical information using NAPRALERT database. Based on the literature review.,the plant was collected from eastern part of the Ooty. *Clerodendrum serratum* is a small tree belonging to family verbenaceae. The plant was taxonomically identified by the botanist Dr.A.Balasubramanian, ABS Botanical Conservation, Research and Training Centre KAARIPATTI, SALEM -636003 T.N., INDIA.

7.2 EXTRACTION OF PLANT [25,26,27]

The leaf of *Clerodendrum serratum* were shade dried at room temperature for 10 to 15 days. After shade drying of leaves, which was converted in to coarse powder form by using the mechanical mixer. Powdered leaf material was defatted using petroleum ether. Defatted plant material was extracted in soxhlet apparatus. Further extract with 90% of ethanol and concentrated by using desiccators for the removal of remaining moisture. The final amount of solid residue was 35% w/w.

7.3 CHEMICALS

- ✓ Ethanol 90%.
- ✓ Petroleum ether.
- ✓ Distilled water.
- ✓ Citric acid 0.1 mg/Spray.
- ✓ Acetylcholine 0.2 % /Spray.

7.4 DRUGS [28,29]

- ✓ Theophylline 200mg/kg.

7.5 SOLVENTS

Ethyl alcohol, Water, CMC (carboxy methyl cellulose)

7.6 PRELIMINARY PHYTOCHEMICAL STUDIES [30]

The pharmacological and therapeutic action of crude drug is determined by the nature of its constituents. Thus the plant species may be considered as a biosynthetic laboratory not only for the chemical compounds e.g. carbohydrates, proteins and fats that are utilized as a food by humans and animals, but also for a magnitude of compounds including alkaloids, flavonoids, glycosides etc. which exert definite physiological effects. These chemical compounds are responsible for the desired therapeutic properties. To obtained these pharmacological effects, the plant materials are used as such in their crude from or may be extracted with suitable solvents to take out the deride components and the resulting principle being employed as therapeutic agents. By considering the above facts, it is necessary to evaluate the nature of extract before evaluating the biological activity of same. We have been selected such extract for pharmacological activity which contain large number of chemical constituents. Hence for this purpose, we have to go for following tests to evaluate the chemical nature of extracts qualitatively.

7.8 TESTS FOR CARBOHYDRATES AND GLYCOSIDES

A small quantity of the extracts was dissolved separately in 4ml of distilled water and filtered. The filtrate was subjected to various tests to detect the presence of Carbohydrates.

Molisch's Test

- Filtrate was treated with 2-3 drops of 1% alcoholic ∞- napthol solutions and 2ml of Con. Sulphuric acid was added along the sides of the test tube. Appearance of brown ring at the junction of two liquids shows the presence of carbohydrates.

- Another portion of the extract was hydrolysed with hydrochloric acid for few hours on a water bath and the hydrolysate was subjected to Legal's and Borntrager's test to detect the presence of different glycosides.

Legal's Test

- To the hydrolysate I ml of pyridine and few drops of sodium nitropruside solutions were added and then it was made alkaline with sodium hydroxide solution. Appearance of pink to red colour shows the presence of glycosides

Bortanger's Test:

- Hydrolysate was treated with choloroform and then the chloroform layer was separated. To this equal quantity of dilute ammonia solution added. Ammonia layer acquires pink color, showing the presence of glycosides.

TEST FOR ALKALOIDS

- A small portion of the solvent free alcohol and aqueous extracts were stirred separately with few drops of dilute hydrochloric acid and filtered. The filtrate was tested with various reagents for the presence of alkaloids.

- Mayer's reagent - Cream ppt
- Dragendroff's reagent - Orange brown ppt.
- Harger's reagent - Yellow ppt
- Wagner's reagent - Reddish brown ppt

TEST FOR PHYTOSTEROL

- The extract was refluxed with solution of alcoholic potassium hydroxide till complete saponification has taken place. The mixture was diluted and extracted with ether. The ether layer was evaporated and the residue was tested for the presence of phytosterol.

Libermann Burchard Test

- The residue was dissolved in few drops of dil. Acetic acid; 3 ml of acetic anhydride was added followed by few drops of Con. Sulphuric acid. Appearance of bluish green color shows the presence of phytosterol.

TESTS FOR FIXED OILS

Spot Test

- Small quantities of various extracts were separately pressed between two filter papers. Appearance of oil stain on the paper indicates the presence of fixed oil. Few drops of 0.5N alcoholic potassium hydroxide were added to small quantity of various extracts along with a drop of phenolphthalein. The mixture was heated on a water bath for 1-2 hours. Formation of soap or partial neutralization of alkali indicates the presence of fixed oils and fats.

TEST FOR GUMS AND MUCILAGES

- Small quantities of the extracts were added separately to 25 ml of absolute alcohol with constant stirring and filtered. The precipitate was dried in air and examined for its swelling properties for the presence of carbohydrates.

TEST FOR SAPONINS

- The extract was diluted with 20ml of distilled water and it was agitated in a graduated cylinder for 15 minutes. The formation of 1cm layer of foam shows the presence of saponins.

TEST FOR PROTEINS AND FREE AMINO ACIDS

Small quantities of the extracts were dissolved in few ml of water and treated with following reagents.

- Appearance of red color shows the presence of protein and free amino acid
- Appearance of purple color shows the presence of proteins and free amino acid
- Equal volumes of 5% sodium hydroxide solution & 1% copper sulphate solution was added.

Appearance of pink or purple color shows the presence of proteins and free amino acids.

TEST FOR PHENOLIC COMPOUNDS AND TANNINS.

Small quantities of the extracts were taken separately in water and test for the presence of phenolic compounds and tannins was carried out with the following reagents.

1. Dil. Ferric Chloride solution (5%) – Violet Color.
2. 1% solution of gelatin containing 10% sodium chloride – White ppt
3. 10% lead acetate solution – White ppt

TEST FOR FLVAVONOIDS

1. **With Aqueous Sodium Hydroxide Solution**

   Blue to violet color (anthocyanins) Yellow color (flavones), yellow to orange (flavonones)
2. **With Concentrating Sulphuric Acid**

   Yellow orange color (anthocyanins) yellow to orange color (flavones) orange to crimson (flavonones)
3. **Shinoda's Test**

Small quantities of the extract were dissolved in alcohol, to them piece of magnesium followed by Conc. Hydrochloric acid drop wise added and heated. Appearance of magenta color shows the presence of flavonoids.

TLC METHOD

Aluminium sheets and glass backed TLC plates were used for the isolation of compounds. The plates were divided in to size of 10 cm x 1.5 cm. A light pencil was drawn 1cm from the bottom and top edge of the chromatographic plate. 6mg of column fraction sample was dissolved in 60 ml of absolute methanol and placed as preparatory on two TLC plates (10x1.5 cm) using a 10 micro litre of capillary which delivers approximately 10 microgram spot on to the place until each plate contains 150 microgram and subsequently placed in the eluting solvent. (Chloroform:methanol:water (5:4:1)) in a TLC tank which was fill to a depth of 0.5 cm the solvent migrated upwards on to the TLC plate until the pencil line drawn across the top edge (solvent front) was reached. The plates were removed from the chamber and air dried. A portion of the plates (1 cm) was cut off using a glass cutter and sprayed with a detecting reagent (diphenyl ethantamine (glycosides)) in order to visualize the constituents on the eluted plates after heating for three minutes at 110^{o} c in on oven. The plates were also visualized under UV light at 360 nm and 254 nm and the fluorescence (360 nm) or quenching (254 nm). Compounds were marked and the spot (layer) were out lined with the pencil.

Distance from origin to the point of maximum intensity

RF value =

Distance from origin to the solvent front

Sample 1: 5/7 = 0.7142.

Sample 2: 4.7/7 = 0.6714.

Report

The RF value of sample 1 and sample 2 were coinciding with standard values.

RF value for sample 1 = 0.7142

RF value for sample 2 = 0.6714.

8-ACUTE ORAL TOXICITY STUDIES

8.1 PROCEDURE[31]

8.1.1 Preparation of animals

The animals are randomly selected, marked to permit individual identification, and kept in their cages for at least 5 days prior to dosing to allow for acclimatisation to the laboratory conditions.

8.1.2 Preparation of doses

In general test substances should be administered in a constant volume over the range of doses to be tested by varying the concentration of the dosing preparation. Where a liquid end product or mixture to be tested however, the use of the undiluted test substances, i.e. at a constant concentration, may be more relevant to the subsequent risk assessment of the substances, and is a requirement of some regulatory authorities. In either case, the maximum dose volume for administration must not be exceeded. The maximum volume of the liquid that can be administrated at one time depends on the size of the test animal. In rodents, the volume should not normally exceed 1ml/100 gm of body weight. However in the case of aqueous solution 2ml/100 gm body weight can be considered. With respect to the formulation the dosing preparation, by use of an aqueous solution/ suspension/ emulsion is recommended wherever possible, followed in order of preference by solution/suspension/emulsion in oil ex. (Corn oil) possible solution in other vehicle. For vehicles other then water the toxicological characteristics of the vehicle should be known. Doses must be prepared shortly prior to administration unless the stability of the preparation over the period during which it will be used is known and shown to be acceptable.

8.1.3 Administration of the dose

The test substance is administered in a single dose by gavages by using a stomach tube or suitable incubation cannula. In the unusual circumstance that a single dose is not possible, the dose may be given in small fraction over period not exceeding 24 hours.

Animal should be fasted prior to dosing (e.g. with rat, food but not water should be withheld over night, with the mouse food but not water should be withheld for 3-4 hours) following the period of fasting, the animal should be weighed and the test substances is administered. After the substance has been administered, food may be withheld for a further 3-4 hours in rats or 1-2 hours in mice. Where a dose is administered infraction over a period it may be necessary to provide the animals with food and water depending on the length of period.

8.1.4 Number of the Animal and Dose Levels

Three animals are used for each step. The dose level to be used as the starting dose is selected from one hour of four fixed levels, 5, 50, 300, 2000 mg/kg body weight. The starting dose level should be that which is most likely to produce mortality in some of the dosed animals.

When available information suggests that mortality is unlikely at the highest starting dose level (2000 mg/kg body weight), then a limit test should be conducted. When there is no information on a substance to be tested, for animal welfare reasons it recommended using the starting dose of 300 mg/kg body weight.

The time interval between treatment groups is determined by the onset, duration, and severity of toxic signs. Treatment of animals at the next dose should be delayed until one is confident of survival of the previously dosed animals.

Exceptionally, and only when one justified by specific regularity needs the use of additional upper dose level 5000 mg/kg body weight may be considered. For reason of animal welfare concern, testing of animals in GHS category 5 ranges (2000 – 5000 mg/kg) is discouraged and should only be considered and when there is a strong likelihood that results of such a test have direct relevance for protecting human or animal health or the environment.

8.1.5 Limit test:

The limit test is primarily used in situations where the experimenter has information indicating that the test material is likely to be nontoxic, i.e., having toxicity only above regulatory limit doses. Information about the toxicity of the test material can be gained from knowledge about similar tested compounds or similar tested mixtures are products, taking into consideration the identity and percentage of components known to be toxicological significance.

A limit test at one dose level of 2000 mg/kg body weight may be carried out with six animals (3 animals per step). Exceptionally a limit test at one dose level of 5000 mg/kg may be carried out with 3 animals. If test substance-related mortality is produced, further testing at the next lower level may need to be carried out.

8.1.6 Test report:

- Tabulation of response data and dose level for each animal (i.e. animal showing signs of toxicity including mortality; nature, severity, and duration of side effects);
- Tabulation of body weight and body weight changes;
- Individual weights of animals at the day of dosing, in weekly intervals thereafter, and at the time of death or sacrifice;
- Date and time of death if prior to scheduled sacrifice;
- Time course of onset of signs of toxicity, and whether these were reversible for each animals;
- Necropsy findings and histopathological findings for each animal.

9-SCREENING METHODS FOR ANTI ASTHMATICS

9.1 ANIMALS

Adult male albino rats, weighing 150 – 200 g were used for the present study. Animals were acclimated for 15 days in our disease free animal house prior to the start of the experiment. The animals were kept in clean and dry plastic cages, with 12 h light: 12 h dark cycle at $25+2^0$ c temperature and 45 – 60% relative humidity. Animals were given free access to standard feed and water and *libitum*. For experimental purpose the animals were kept on overnight fasting but allowed free access to water. The research conducted under the guidelines of CPCSEA and approved by Institutional Animal Ethics Committee in ref. No. M.Pharma/2013/09.

INVIVO METHOD[32,33,34,35]

9.2 EXPERIMENTAL DESIGN – I[36,37,38,39]

Overnight fasted Rats were divided into six groups

1. Inducer control (IC) = Acetylcholine + Citric acid (0.2% spray),
2. STD received Theophylline (200 mg/kg)
3. AECS (100mg/kg) (200mg/kg),
4. NAECS (200mg/kg) (400mg/kg),p.o. Bronchospasm was induced in rats by exposing them to Acetylcholine & Citric acid (0.2% spray) produced by an ultra sound nebulizer in an aerosol chamber (24*14*24 cm) made of Perspex glass. The time required for appearance of pre convulsive dyspnoea caused by the Acetylcholine & Citric acid (0.2% spray) was recorded for each animal. Prior to drug treatment, each animal was placed on Histamine chamber and exposed to Acetylcholine + Citric acid 0.2% aerosol. The preconvulsive time (PCT), i.e the time of aerosol exposure to the onset of dyspnoea leading to the appearance of

convulsion, was noted. As soon as the preconvulsion dyspnoea (PCD) was noted, the animals were removed from the chamber and placed in fresh air to recover as basal value. Rats were then allowed to recover from dyspnoea for 24 hrs. After 24 hrs, the animals of STD received Theophylline (200 mg/kg), AECS (100mg/kg) (200mg/kg), NAECS (200mg/kg) (400mg/kg). These animals were again subjected to Acetylcholine + Citric acid 0.2% aerosol later at an interval of 1hr, 4hrs, and 24 hrs to determine preconvulsion time (PCT). The protection offered by the treatment was calculated by using the following formula

Percentage protection = $(1 - T_1/T_2) * 100$

Where, T_1 = the mean of PCT before administration of test drugs, and

T_2 = the mean of PCT after administration of test drugs at 1 hr, 4 hrs and 24 hrs.

9.3 STATISTICAL ANALYSIS

All the values were expressed as mean $^{+}$. SEM. The results were analyzed for statistical significance by using one-way ANOVA followed by Dunnett's test. $P<0.05$ was considered significant.

PHARMACOLOGICAL STUDIES

INVITRO METHOD

9.4 INDUCTION OF ASTHMA[40,41,42,43,44]

Asthma was induced by citric acid 0.1 mg and acetylcholine 0.2 mg in the form of spraying in alternative days for two weeks. The extract of *c.serratum* and Theophylline was administered by oral route for two weeks. At the end of the treatment, all of the rats were sacrificed and Lungs Blood Samples were taken out, fixed then histopathological studies were followed.

9.5 EXPERIMENTAL DESIGN – I

24 male adult rats were randomly divided into 6 groups:

- ✓ Asthma group received a normal diet (A)
- ✓ Asthma group treated with Theophylline (200 mg/kg b.w.) (T)
- ✓ Asthma group which received AECS (100 mg/kg b.w.) (P1)
- ✓ Asthma group which received AECS (200 mg/kg b.w.) (P2)
- ✓ Asthma group which received NAECS (200 mg/kg b.w.) (E1)
- ✓ Asthma group which received NAECS (400 mg/kg b.w.) (E2)

9.6 PARAMETERS FOR INVESTIGATION

The other major type of blood cells are the white blood cells (WBC's), which are also referred to as leukocytes. There are many more RBC's than there are WBC's. For every leukocyte present in a sample there will normally be 600 to 700 RBC's. The major role of the white blood cells is to defend the body against invading organisms such as bacteria, viruses, and fungi. There are different types of leukocytes, and a white blood count (WBC) is a total of all the various kinds. The normal range for a WBC count in the dog would be between 6,000 and 17,000 per microliter, and in the cat, 4,900-20,000/µ l. The number of WBC's is typically elevated when the body is fighting a severe infection or stressed by metabolic toxins (a patient that was in kidney failure with waste products building up in its body would normally have an elevated WBC). In addition, when extremely excited (if we overly excite or frighten the animal when drawing

the blood sample) white blood cells will be released into the blood and the levels will rise. The WBC count will be lower than normal, if an animal has been weakened from a prolonged, debilitating disease and in some viral infections.

WBC's are divided into two groups depending on how they react to the stains that are used to better observe them under a microscope. There are granulocytes (they are WBC's with granules that absorb the stain) and the agranulocytes (those that do not absorb the stain). The granulocytes include the neutrophils, eosinophils, and the basophils, while the agranulocytes are the lymphocytes and monocytes

9.7 HISTOPATHOLOGICAL EXAMINATION[45,46,47,48]

After 14 days experimental period and the last blood sampling, the whole Lungs were removed after sacrificing the animal and were fixed in 10% formalin for histopathological examination. Sections were cut and stained by hematoxylin and eosin (H&E) for histological examination.

9.8 STATISTICAL ANALYSIS

All the values were expressed as mean $^{+}$. SEM. The results were analyzed for statistical significance by using one-way ANOVA followed by Dunnett's test. $P<0.05$ was considered significant.

10-RESULTS AND DISCUSSIONS

Table-2

10.1 PHYTOCHEMICAL CONSTITUENTS OF DIFFERENT EXTRACTS OF LEAVES OF *CLERODENDRUM SERRATUM*

PhytoChemical Constituents	Aqueous extract	Non aqueous extract
Carbohydrates	-	-
Glycosides	-	-
Alkaloids	-	-
Flavonoids	+	+
Phenols	+	+
Fixed oils	-	-
Steroids	+	+
Saponins	-	-
Gums & mucilage	-	-
Proteins & free amino acids	-	-

(+) → Positive

(-) → Negative

10.2 DATA SHOWING ACUTE ORAL TOXICITY

ortality result of sighting study starting dose in main study is decided and carried out with six animals per dose level
00mg/kg).Based on the mortality result on 14^{h} day of observation, the doses for *in vivo* study were selected.

TREATMENT	Mg/kgDOSE	MORTALITY	SEDATION	CONVULSION	URINATION	GESBODY COLOR	LOCOMOTION	WEIGHTBODY
AECS	mg/kg5	-	-	-	-	-	-	-
AECS	50 mg/kg	-	-	-	-	-	-	-
AECS	200mg/kg	-	-	-	-	-	-	-
AECS	1000mg/kg	+	-	-	- mild	-	- mild	-

10.3 ACUTE ORAL TOXICITY NAECS[54]

ortality result of sighting study starting dose in main study is decided and carried out with six animals per dose level
)mg/kg).Based on the mortality result on 14^{h} day of observation, the doses for *in vivo* study were selected.

TREATMENT	Mg/kgDOSE	MORTALITY	SEDATION	CONVULSION	URINATION	BODY COLOR CHANGES	LOCOMOTION	WEIGHTBODY
NAECS	mg/kg5	-	-	-	-	-	-	-
NAECS	50 mg/kg	-	-	-	-	-	-	-
NAECS	300mg/kg	-	-	-	-	-	-	-
NAECS	2000mg/kg	-	-	-	-	-	-	-

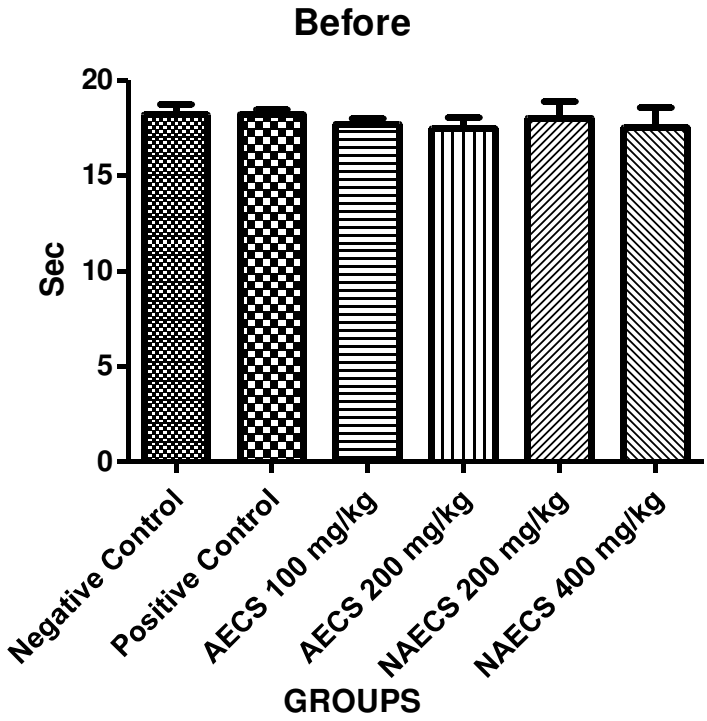
10.4 PRE CONVULSION DYSNOPEA[55,56,57,58,59]

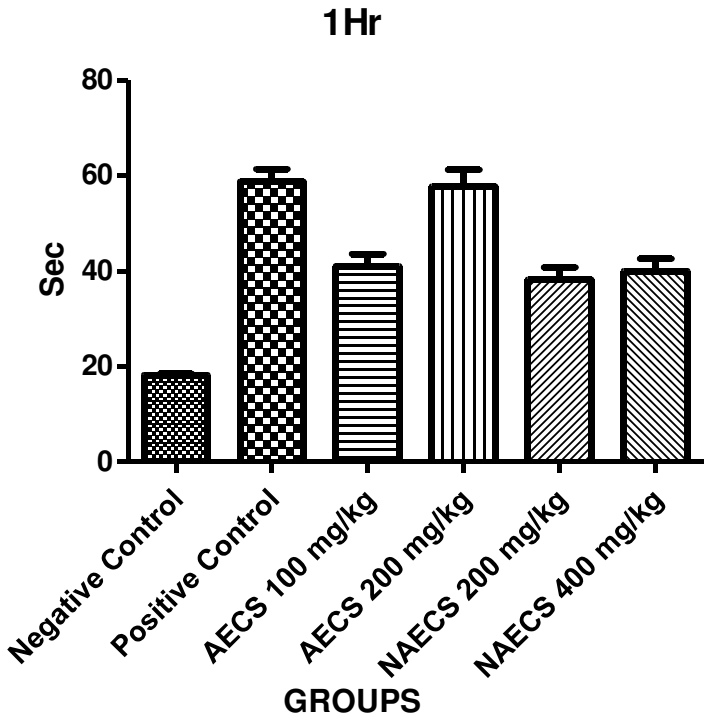
TREATMENT	DOSE	BEFORE	1 Hr	4 Hrs	24 Hrs
Negative control	Ach + Citric acid	18.2 ± 2.2739	18.2 ± 0.1250	17.4 ± 0.091	17.2 ± 0.110
Positive control	Ach + Citric acid + Theophylline	18.2 ± 0.1250	55.8 ± 1.315	64.3 ± 1.548**	34 ± 1.472**
AECS	100 mg/kg	17.5 ± 0.1371	41 ± 1.291	43 ± 1.291	22.8 ± 0.478
AECS	200 mg/kg	17.5 ± 0.2955	57.8 ± 1.750	59.8 ± 1.750**	33 ± 1.225**
NAECS	200 mg/kg	18.0 ± 0.4328	38.3 ± 1.250	40 ± 1.250	21.8 ± 0.478
NAECS	400 mg/kg	17.5 ± 0.5282	40 ± 1.291	41.5 ± 1.258	27.1 ± 0.314

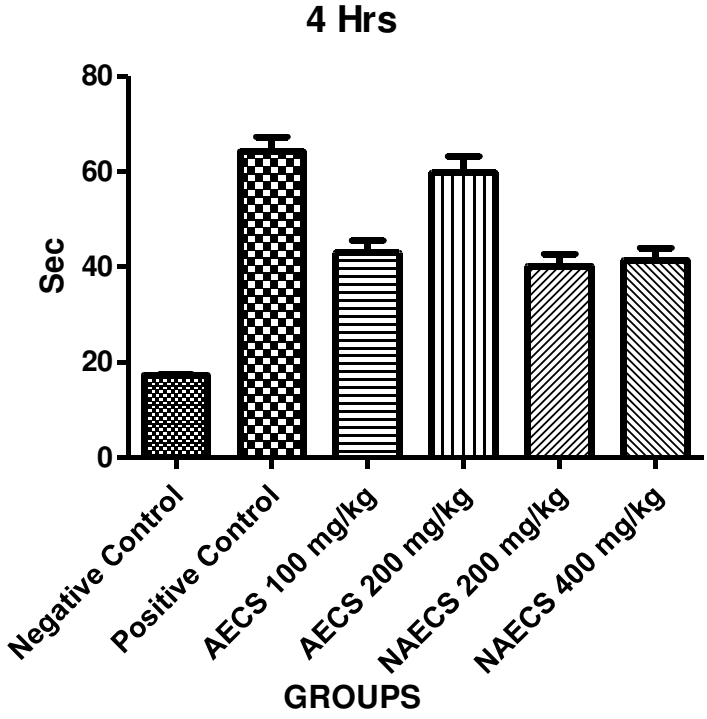
Values are represented as mean ± S.E.M (n=6)

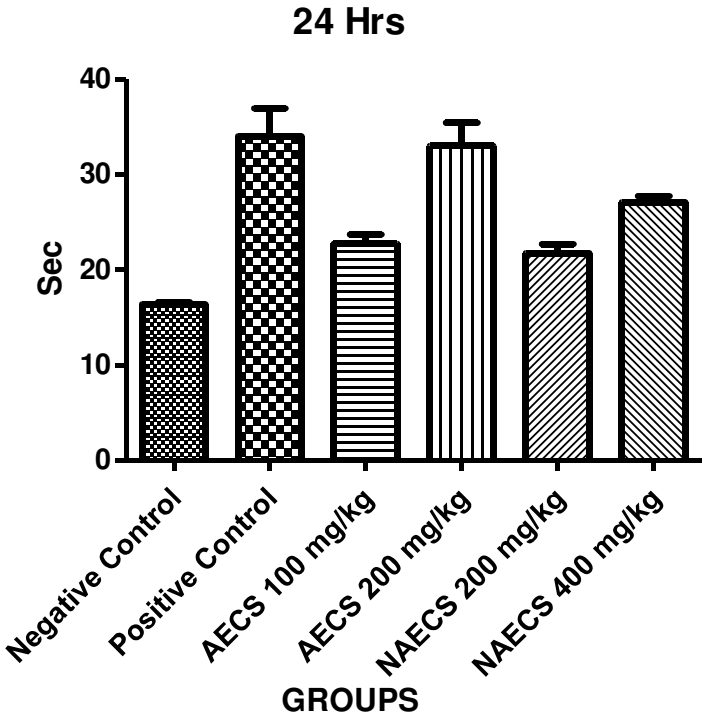
One-way ANOVA followed by Student-Newman-Keuls post test (P< 0.001)

The Ach + Citric acid induced asthma in rats, results have expressed on Table. All the groups of animals were affected in asthma, which indicated PCD were constantly increased, the aqueous extract of *c.serratum* 100 & 200 mg/kg and non aqueous extract of *c.serratum* treated groups 200 & 400 mg/kg were dose dependent manner decreased (P<0.001)**& (P<0.0001)*** (59.8 + 1.750**↓&33 + 1.225**↓), (41.5 + 1.258↓ & 27.1 + 0.314↓) .When compared with control group but positive control have more anti asthmatic activity (P<0.001)**& (P<0.0001)*** When compare to each groups of aqueous extract of *c.serratum* 200 mg/kg have equipotent activity (59.8 + 1.750**↓&33 + 1.225**↓). When compared with positive control. The aqueous extract of *c.serratum* 100&200 mg/kg have been expressed more anti asthmatic action (98.6±1.319↓ & 53.8±0.979↓) (P<0.001) **& (P<0.0001) *** When compared to non aqueous extract of *c.serratum* groups.


Before
20
15
10
5
0
Sec
Negative Control
Positive Control
AECS 100 mg/kg
AECS 200 mg/kg
NAECS 200 mg/kg
NAECS 400 mg/kg
GROUPS


1Hr
80
60
40
20
0
Sec
Negative Control
Positive Control
AECS 100 mg/kg
AECS 200 mg/kg
NAECS 200 mg/kg
NAECS 400 mg/kg
GROUPS


4 Hrs
80
60
40
20
0
Sec
Negative Control
Positive Control
AECS 100 mg/kg
AECS 200 mg/kg
NAECS 200 mg/kg
NAECS 400 mg/kg
GROUPS


24 Hrs
40
30
20
10
0
Sec
Negative Control
Positive Control
AECS 100 mg/kg
AECS 200 mg/kg
NAECS 200 mg/kg
NAECS 400 mg/kg
GROUPS

10.5 ANTI ASTHMATIC ACTIVITY[60,61,62,63,64]

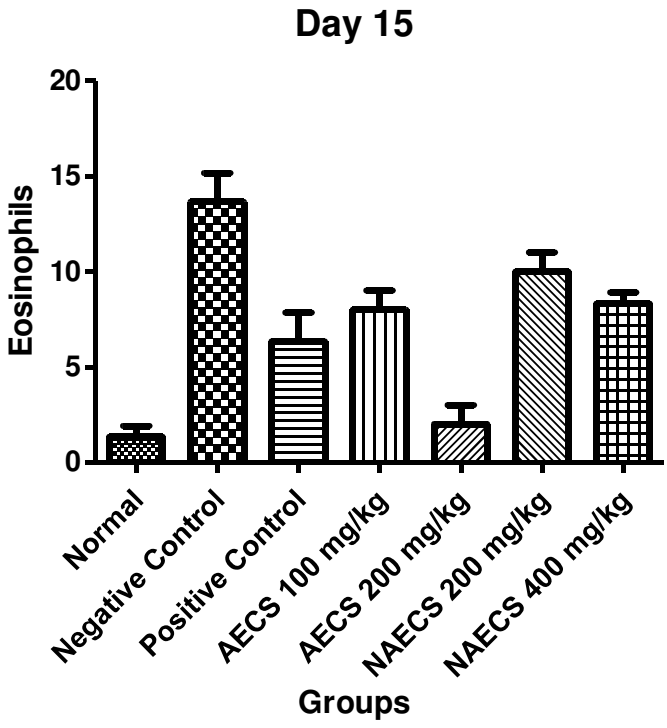
Normal	Negative Control	Positive Control	AECS 100 mg/kg	AECS 200 mg/kg	NAECS 200 mg/kg	NAECS 400 mg/kg
1.33 ± 0.333	13.66 ± 0.881	6.33 ± 0.881**	8 ± 0.577	2 ± 0.577**	10 ± 0.577	8.33 ± 0.333

Values are represented as mean ± S.E.M (n=6)

One-way ANOVA followed by Student-Newman-Keuls post test (P< 0.001)

The effect of Aq. and Non Aq. extract of *c.serratum* Eosinophils on indicated in Table, which is correlated to anti asthmatic action. After the Ach + Citric acid treatment, on day 15 all the groups of animal Eosinophils levels were significantly decreased (13.66 ± 0.881↑, 6.33 ± 0.881**↓, 8 ± 0.577, 2 ± 0.577). The Eosinophils levels were significantly dose dependent manner decreased, after the treatment of Aq. and Non Aq. extract of *c.serratum* 100, 200 & 200, 400 mg/kg (8 ± 0.577, 2 ± 0.577**↓, 10 ± 0.577, 8.33 ± 0.333) *** at 15 th day.

On day 15th the Aqueous extract of *c.serratum* treated groups 100 & 200 mg/kg were Eosinophils levels were more significantly decreased (P<0.001) ** & (P<0.0001)*** when compared with Non Aq. extract of *c.serratum*. The Aqueous extract of *c.serratum* 200 mg/kg have equipotent activity (2 ± 0.577**↓) when compared with Normal group (1.33 ± 0.333). [43,44,45,46,47]


Day 15
Eosinophils
20
15
10
5
0
Normal
Negative Control
Positive Control
AECS 100 mg/kg
AECS 200 mg/kg
NAECS 200 mg/kg
NAECS 400 mg/kg
Groups

10.6 HISTOPATHOLOGICAL REPORT

Figure: 1 Control group

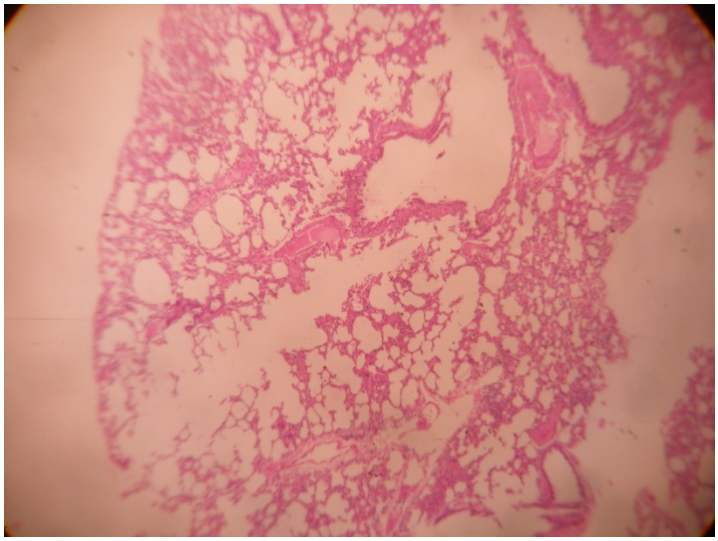
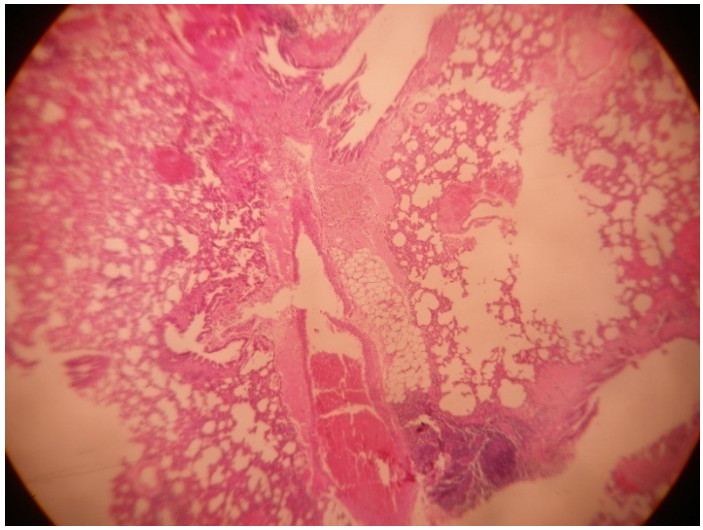


Figure: 2 Control group



IMPRESSION:

The alveoli thickness is normal so free of haemorrhage.

Figure: 3 Negative Control group

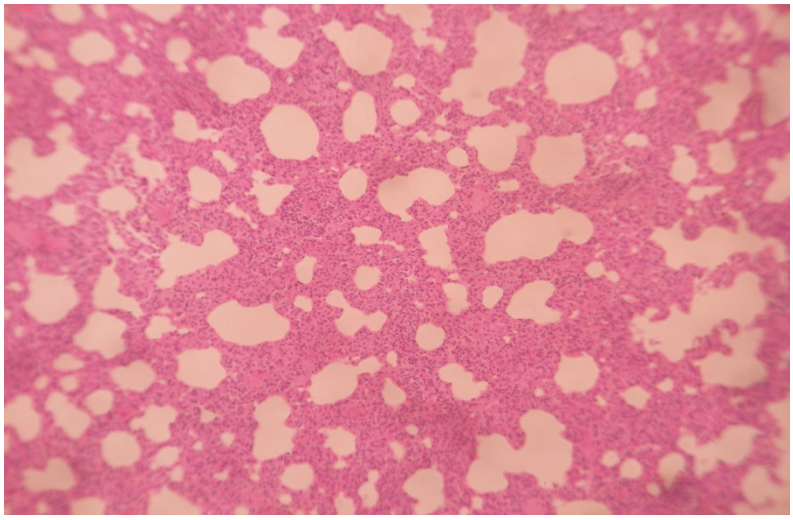
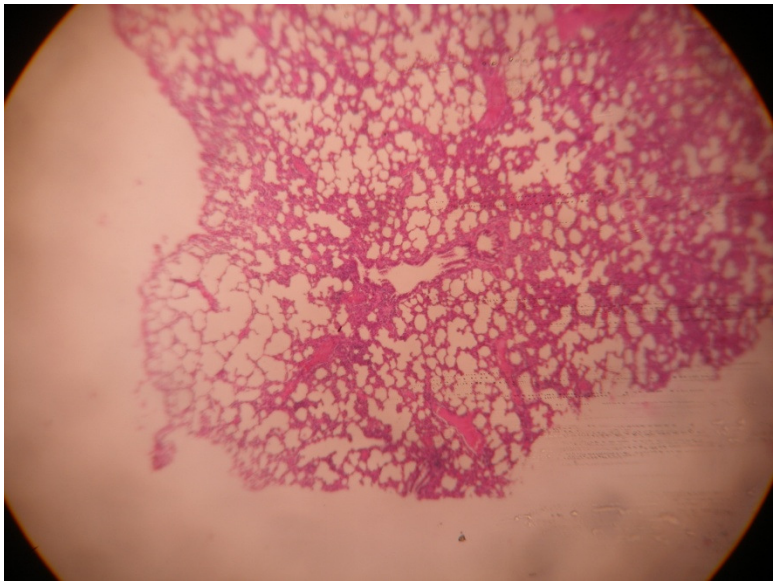


Figure: 4 Negative Control group



IMPRESSION:

Alveolar wall appears thickened in most of the areas when compared to control group.

Figure: 5 Positive Control group

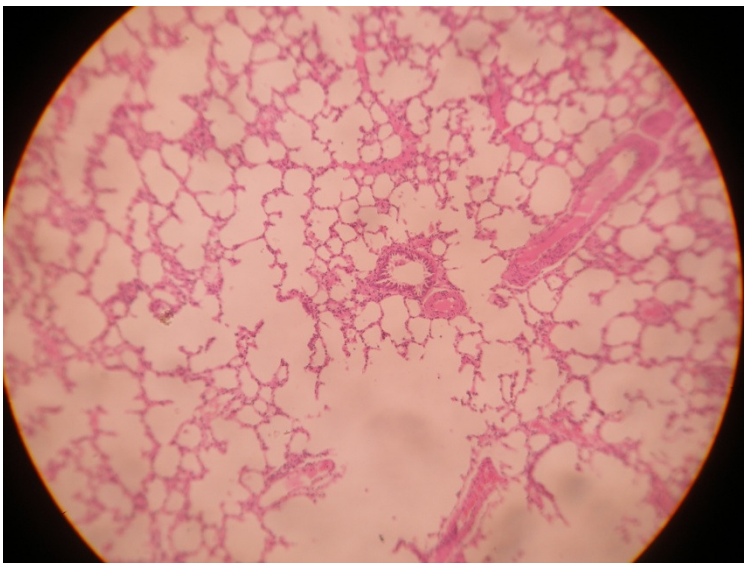
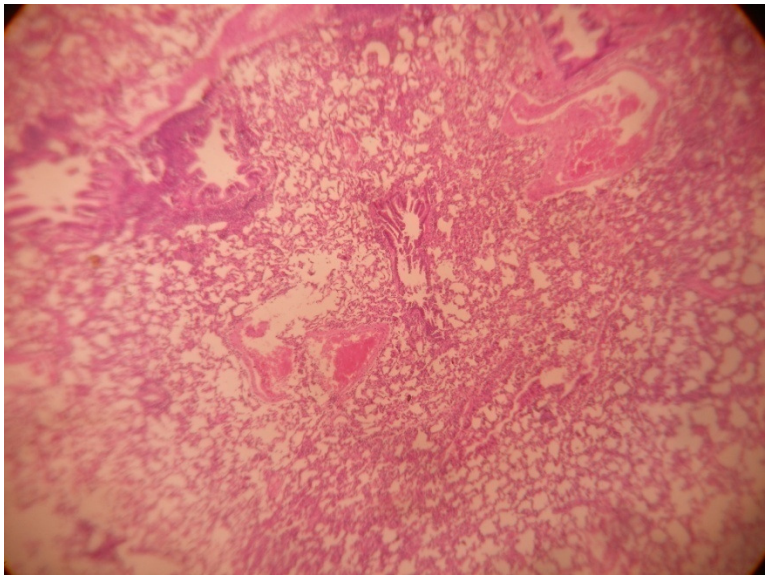


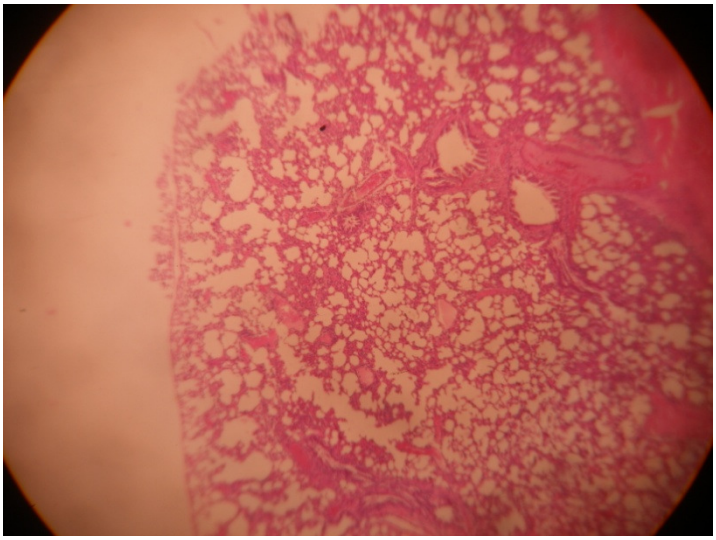
Figure: 6 Positive Control group



IMPRESSION:

Alveolar wall thickness appears normal in both lungs.

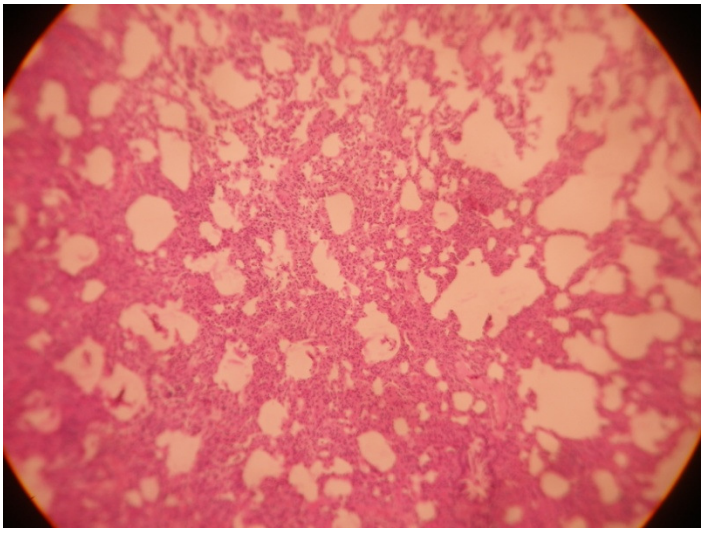
Figure: 7 Aq.Extract 100 mg/kg Treated group



IMPRESSION:

Minimal thickening of the alveolar walls when compared to Control group.

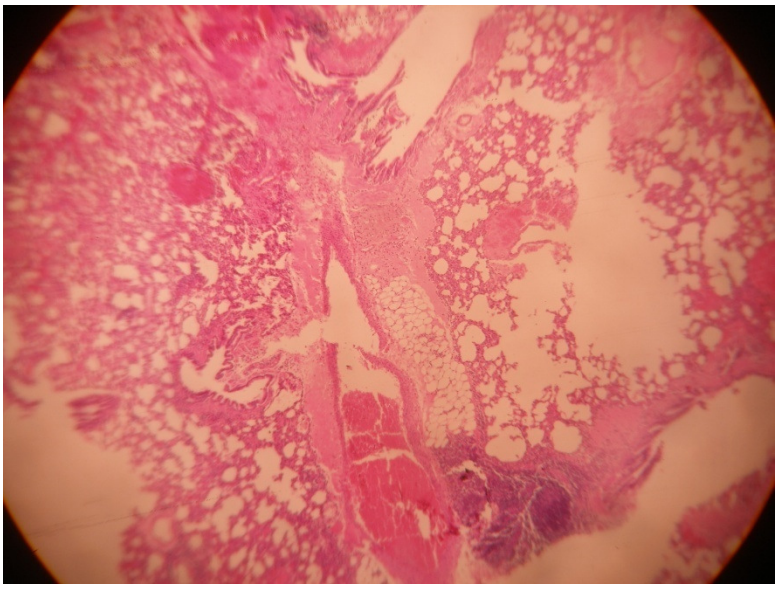
Figure: 8 Aq.Extract 200 mg/kg Treated group



IMPRESSION:

No more thickening of the alveolar walls when compared to Control group.

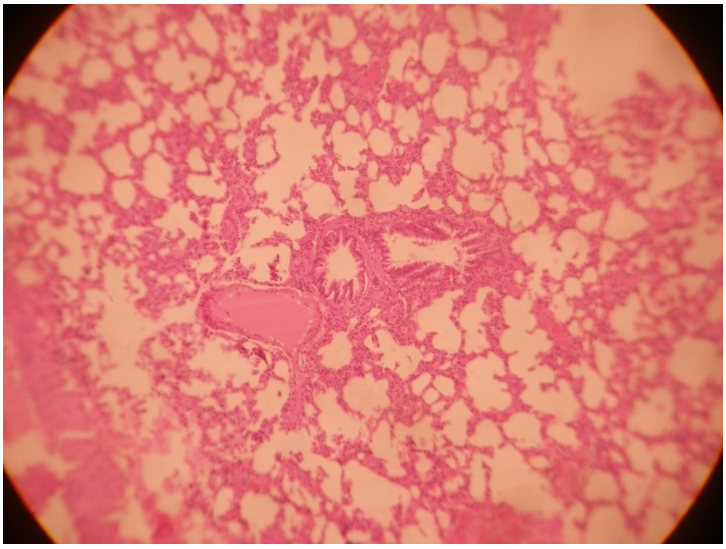
Figure: 8 Non Aq.Extract 200 mg/kg Treated group



IMPRESSION:

More thickening of the alveolar walls when compared to Control group and slightly less thickening walls when compared to Negative control group.

Figure: 8 Non Aq.Extract 400 mg/kg Treated group



IMPRESSION:

Minimal thickening of the alveolar walls when compared to Control group.

DISCUSSIONS

Asthma is common respiratory disease. The morbidity and the mortality of the disease is increasing and making a global concern. The syndrome of bronchial asthma is characterized by wide spread narrowing of the bronchial tree due to contraction of the smooth muscle in response to multiple stimuli resulting in the release of chemical mediators such as Ach and Citric acid. In the presence study *c.serratum*..... Significantly inhibited the Ach and Citric acid induced anti asthmatic properties of the plant.

Ach and Citric acid induced Bronchoconstriction is the traditional immunological model of the antigen induced air way obstruction. Ach and Citric acid when inhaled causes hypoxia and leads to convulsion in rats and causes very strong smooth muscle contraction, profound hypotension, capillary dilation in cardio vascular system a prominent effect caused by histamine leads to severe Bronchoconstriction in rats that causes asphyxia and death. Bronchodilator can delay the occurrence of these symptoms. The results of the study confirmed the bronchodilator properties of the plant, justifying its traditional claim in the treatment of asthma.

Drugs effective in the asthma are mostly steroidal and also flavonoids in nature. Phytochemical profile of the plant reveals the presence flavonoids (Apigenin), steroidal nucleus (α-spinasterol) in the form of triterpenoids. The anti asthmatic activity showed by leaves of the plant extract (Apigenin and α-spinasterol) may be because of the chemical moieties. However this claims demands for further research and studies are in fact underway to isolate and characterized the active principles responsible for the anti asthmatic activity.

11-CONCLUSION

On the basis of the result in these experiments, this may be stated that the aqueous extract of *Clerodendrum serratum* 200mg/kg has a beneficial effect in asthmatic patients. It will reduce the **alveolar thickness** and **Eosinophils** counts in blood further studies are required to purify the active principle and to study the molecular mechanism of the exact pathway.

Non aqueous extract of *Clerodendrum serratum* (400mg/kg) having fewer amounts of steroids and flavonoids because it will filter the all compounds and reduce the quantity of chemical moieties.

But aqueous extract of *Clerodendrum serratum* (200mg/kg) having higher amount of chemicals when compare to Non aqueous extract of *Clerodendrum serratum* (400mg/kg) so it will produce anti asthmatic at equal to standard drug.

In lungs alveolar thickness reduced by the AECS 200mg/kg as equal to standard drug and also it's same as normal lungs.

So aqueous extract of *Clerodendrum serratum* having good beneficial effect for the asthmatic patients.

This information's will be useful for the development of alternative method rather than anti asthmatic agents (Inhalers, Tablets, Injections, Nasal sprays, Respules) for the treatment of Asthmatic patients. This will minimize the wheezing and or asthmatic symptoms.

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COMMENTS

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